Initial reports of FLO2 described up-regulation during regeneration in goldfish optic neurons and co-purification with lipid rafts in detergent resistant membrane fractions. We tested available loss- and gain-of-function alleles of *flo2* and found it to be both necessary and sufficient to inhibit wound-mediated activation of several wound response genes. FLO2 requires post-translational modifications to maintain its association with the cell membrane and to direct signaling events. Reduced function of members of the Src Kinase family, using a small molecule inhibitor, shows a dose dependent activation of the wound response reporters in all epidermal cells. Based on results from other labs, this suggests that Scr phosphorylation of FLO2 may regulate how it limits the spread of epidermal wound responses. Understanding the coordinate roles of FLO2 sensing an injury and orchestrating downstream signaling pathways will further our understanding of the wound healing process.

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Program/Abstract # 376
Grainy head phosphorylation is essential for wound-dependent regeneration of an epidermal barrier but dispensable for embryonic barrier development
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Grainy head (GRH) is a key transcription factor that is required for the expression of genes mediating epidermal barrier formation and maintenance. The physiological function of GRH is highly conserved across diverse animal species. However, it is unknown how GRH activity is modulated during development and tissue repair. Here, we show that GRH is directly regulated by extracellular signal-regulated kinase (ERK) phosphorylation. The ERK phosphorylation is required for wound-induced expression of GRH target genes in epidermal cells. Our biochemical analyses have revealed that Serine 91 is the principal residue in GRH that is phosphorylated by ERK. Although mutations of the ERK phosphorylation sites in GRH do not impair its DNA binding affinity, the ERK sites in GRH are required to activate *Dopa decarboxylase* (*Ddc*) and *misshapen* (*msn*) epidermal wound enhancers after epidermal wounding. These data indicate that the phosphorylation sites are critical for epidermal barrier repair. However, GRH with mutated ERK phosphorylation sites can still promote barrier generation during embryonic epidermal development, suggesting that ERK sites are dispensable for the physiological function of GRH in establishing epidermal barrier integrity. These results provide mechanistic insight into how tissue regeneration can be initiated by post-translational modification of a key transcription factor that normally mediates the developmental generation of that tissue.

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Program/Abstract # 377
Nerve-dependent gene expression in the epidermis of regenerating salamander limbs
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Salamander limb regeneration is dependent upon an intact nerve supply and a specialized wound epidermis (WE) termed the apical epidermal cap (AEC). Regeneration fails if either of these structures is removed from the injured limb as both structures are thought to supply growth cues to the regenerating tissue. Early during limb regeneration the WE is invaded by nerve fibers which induce the expression of genes restricted to the AEC including *sp9*, *dlx2*, and *fgf2*. However, the majority of downstream nerve targets that control regeneration have not been identified. To investigate this problem we used microarray analysis to compare mRNA transcript abundances between three different types of epidermis: 1) the WE of regenerating forelimbs, 2) the WE of denervated regenerating forelimbs, and 3) the WE of healing flank wounds (which do not require nerves for repair). Tissues were collected from Mexican axolotls prior to injury and at 1, 3, and 7 days following injury. Using the new generation of axolotl microarrays gave us the potential to interrogate ~20,000 transcripts and as a result, many differences between the three types of WE were identified. Specifically, we found genes unique to the innervated WE of the regenerating limb, which make them prime candidates for signaling the maintenance of proliferation in blastemal cells by the AEC. The transcriptional profiles identified in this study are crucial for understanding the role of the nervous system in limb regeneration.

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Program/Abstract # 378
Epiplakin1 (Eppk1) marks the cholangiocytes and transit amplifying cells in injured liver
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In the liver, oval cells appear in response to injury and are considered as transit amplifying cells. The current understanding is that these cells play a role in the maintenance of the homeostasis of liver upon injury by their potential of differentiation to give rise to both hepatocytes and cholangiocytes, similar with hepatoblasts that are known as embryonic hepatic progenitor cells. However, their exact role and origin remain unclear. The lineage tracing study is considered as a powerful tool in order to identify the stem cell or progenitor cells for the investigation of their role. We search for useful marker to identify oval cells and trace their progenies. We have previously reported that Eppk1, a plakin family gene known as a cytolinker protein, marks pancreatic progenitor cells, and at E8.5, Eppk1 is expressed in the foregut endoderm, which gives rise to the pancreas and liver (Yoshida T et al., 2008, 2009). We hypothesize that Eppk1 is a useful candidate marker of progenitor cells, not only in the pancreas but also the liver. To test this hypothesis, we analyzed the expression patterns of Eppk1. We found that Eppk1 marks oval cell populations in the adult injured liver. In developing embryos, Eppk1 expression was observed in the cholangiocyte progenitor population. After birth, in normal and injured adult liver, Eppk1 expression was observed in the cholangiocytes and oval cells, respectively. Taken together, we propose that Eppk1 is a useful marker to identify oval cell.

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Program/Abstract # 380
Numb plays a critical role in satellite cell mediated muscle repair
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Numb is a cytoplasmic adaptor protein that has a role in cell fate determination and marks Notch1 for degradation by the proteasome.